CALIFORNIA STATE

MOSQUITO-BORNE VIRUS SURVEILLANCE & RESPONSE PLAN

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CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

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Introduction

California has a comprehensive mosquito-borne disease surveillance program that has monitored mosquito abundance and mosquito-borne virus activity since 1969. Surveillance and interagency response guidelines have been published previously by the California Department of Health Services (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). The detection of West Nile (WN) virus in New York, a virus never recognized prior to 1999 in the Western Hemisphere, has prompted a review of existing guidelines to ensure that WN will be detected by the surveillance system. In addition to WN virus, California is at risk for introduction of other highly virulent mosquito-borne viruses, such as, Japanese encephalitis, dengue, yellow fever, Rift Valley fever, and Venezuelan encephalitis viruses. If an existing or introduced virus is detected, it is critical that local and state agencies are prepared to respond in a concerted effort to protect people and animals from infection and disease. The current document describes an enhanced surveillance and response program for the State of California. Its contents represent the collective effort of the California Department of Health Services (DHS), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD) and Berkeley (UCB).

Background

Mosquito-borne viruses belong to a group of arthropod-borne viruses referred to as arboviruses (for **ar**thropod-**bo**rne). Although 12 mosquito-borne viruses are known to occur in California, only western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) have caused significant outbreaks of human disease. Consequently, the California Arbovirus Surveillance Program emphasizes forecasting and monitoring the temporal and spatial activity of SLE and WEE. Both viruses are maintained in nature in wild bird-mosquito cycles, and therefore are not dependent upon infections of humans or domestic animals for their persistence. In California, surveillance and control activities focus on this cycle, which involves primarily the western encephalitis mosquito, *Culex tarsalis*, and birds such as house finches and sparrows.

Immature stages (called larvae and pupae) of *Culex tarsalis* can be found throughout California in a wide variety of aquatic sources, ranging from clean to highly polluted waters. Most such water is associated with irrigation of agricultural crops or urban wastewater. Other mosquito species, such as *Culex quinquefasciatus*, may play an important role in the SLE transmission cycle in urban and suburban areas in southern California. *Ochlerotatus melanimon*, a floodwater mosquito, plays a role in a secondary transmission cycle of WEE involving rabbits.

Mosquito control is the only practical method of protecting people and animals from SLE or WEE infections. There are no known specific treatments or cures for diseases caused by these viruses. Vaccines are not available for general public use and because most human infections do not result in clinical disease, there is little stimulus for their development and use. Infection by WEE virus tends to be most serious in very young children, whereas infection caused by SLE virus affects elderly people most seriously. WEE can be an important disease in horses and emus. There is an effective WEE vaccine available to protect horses.

Mosquito-borne disease prevention strategies must be based on a well-planned, area-wide integrated pest management (IPM) based program. The primary components of an IPM program include education, surveillance, and mosquito control.

Education

Residents, farmers, and duck club owners can play an important role in reducing the number of adult mosquitoes by eliminating standing water that may support the development of immature mosquitoes. For instance, residents can help by properly disposing of discarded tires, cans, or buckets; emptying plastic or unused swimming pools; and unclogging blocked rain gutters around homes or businesses. Farmers and ranchers can ensure irrigation practices do not allow standing water for extended periods, and duck club owners can work with mosquito control agencies to determine appropriate flooding schedules. Education regarding personal protective measures such as the use of insect repellents or the wearing of long-sleeved clothing will help reduce exposure to mosquitoes. Equally important is the education of the medical community to recognize the symptoms of WEE and SLE and request proper laboratory testing for their confirmation. Public health officials need to be alerted if a mosquito-borne viral disease is detected, especially if the public health risk is high.

Surveillance

Surveillance includes the monitoring of immature and adult mosquito abundance and detecting virus activity by testing (1) mosquitoes, (2) sentinel chickens and wild birds, (3) horses, and (4) humans for infection. Surveillance must include not only the monitoring of mosquito-borne viruses known to exist in California, but also the detection of newly introduced viruses.

Mosquito Abundance

A "dipper," or long-handled ladle, is used to collect water samples from most mosquito sources. The number of immature (larvae and pupae) mosquitoes per "dip" can then be estimated. In most local mosquito control agencies, technicians search for new sources and inspect known habitats for mosquitoes on a seven to 14-day cycle. These data are used to direct control operations. Maintaining careful records of immature mosquito occurrence, developmental stages treated, source size, and control effectiveness can provide an early warning to forecast the size of the adult population.

Four adult mosquito-sampling methods are currently in use in California: New Jersey light traps, carbon dioxide-baited traps, gravid (egg-laying) traps, and resting adult mosquito collections. The advantages and disadvantages of these sampling methods, and guidelines for the design, operation, and processing of the traps, are summarized in Appendix A. Monitoring the abundance of adult mosquito populations provides important information on the effectiveness of larval control efforts and the size of the vector population. Adult mosquito abundance is a key factor when evaluating the risk of disease transmission.

Mosquito Infections

Early detection of virus activity may be accomplished by testing adult mosquitoes for virus infection. Because *Culex tarsalis* is the primary vector of both WEE and SLE in California, surveillance efforts emphasize the testing of mosquitoes of this species. Female mosquitoes are trapped, usually using carbon dioxide-baited traps, and pooled into groups of 50 females each for submission to the laboratory at Davis Arbovirus Research Unit (DARU), which is part of the UC Davis Center for Vector-borne Disease Research. Procedures for processing mosquitoes for virus infection are detailed in Appendix B. The current surveillance system is designed to detect WN virus and other vector-borne viruses, in addition to SLE and WEE. Although generally less sensitive than sentinel chickens, mosquito infections may be detected earlier in the season than chicken seroconversions and therefore provide an earlier warning of virus activity. Testing adult mosquitoes for infection is one of the best methods to detect newly introduced mosquito-borne viruses that would not otherwise be expected to be present in the state. Sampling mosquito species other than *Cx. tarsalis* may be necessary to detect the introduction of viruses that do not have a primary avian-*Culex* transmission cycle.

Avian Infections

Detection of transmission of arboviruses in bird populations can be accomplished by using caged chickens as sentinels and bleeding them routinely to detect viral antibodies (seroconversions) or by collecting and bleeding wild birds to detect viral antibodies in populations. In California, flocks of ten chickens are placed in small pens in a location where mosquito abundance is known to be high or where there is a history of virus activity. Each chicken is bled biweekly by pricking the comb and collecting blood on a filter paper strip. The blood is tested at DHS' Viral and Rickettsial Diseases Laboratory for antibodies to SLE and WEE. Some agencies conduct their own testing, but send positive samples to DHS for confirmation and official reporting. Because SLE cross-reacts with WN in antibody testing, serum drawn from the first chicken from a flock that is positive for SLE is also tested for WN, and possibly other viruses, at UCD. Frequent bleeding of carefully placed flocks of sentinel chickens provides the most sensitive and cost-effective method to monitor both WEE and SLE activity. Because chickens are continuously available to host-seeking mosquitoes, they are usually exposed to more mosquitoes than can be collected by trapping, especially when adult mosquito abundance is low. Sentinel housing, bleeding instructions, and testing protocols are provided in Appendix C.

Virus activity in wild bird populations also can be monitored by bleeding young (hatching year) birds to detect virus infection, or bleeding older birds to determine if the prevalence of the virus in the region has changed. Seroprevalence can be detected in banded recaptured birds. In contrast to the convenience of using sentinel chickens, the repeated collection and bleeding of wild birds generally is too labor intensive, technically difficult, and expensive for routine surveillance activities by local mosquito control agencies. In addition, the actual place where a wild bird became infected is rarely known, because birds usually are collected during daylight foraging flights and not at nocturnal roosting sites where they are most frequently bitten by mosquitoes.

In 2000, surveillance for WN and other mosquito-borne viruses in dead crows was initiated because these birds have been shown to provide an early warning that WN is circulating in a region. Although there is currently no evidence of the presence of WN in California, WN could be imported to California through interstate or international transport of birds, mosquitoes, or mammals. Another possible source for introduction is by interchange of infected birds between the Atlantic, Mississippi, and Pacific flyways. In collaboration with many local, state, and federal agencies, crows that meet certain criteria are being tested for a panel of mosquito-borne viruses. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

Equine Infections

Currently, equine disease due to WEE is not a sensitive indicator of epizootic (the occurrence of infections in animals other than humans) WEE activity in California because of the widespread vaccination of equines (horses, donkeys, and mules) against WEE infections. If confirmed cases do occur, it is a strong indication that WEE is active in that region of the State. Veterinarians are contacted annually by DHS and the California Department of Agriculture (CDFA) to ensure that equines are vaccinated and to describe diagnostic services that are available in the event of a suspected case of WEE or WN fever. Besides WEE and WN, other mosquito-borne viruses may also cause encephalitis in horses, and consequently, testing of equine specimens has been expanded to include other viruses (see Appendix E).

Human Infections

Human cases are an insensitive surveillance indicator of virus activity because most human infections (>99%) have no, or only mild, symptoms. When severe cases do occur (e.g. encephalitis or aseptic meningitis), physicians may not establish a definitive diagnosis of an arboviral disease. Rarely are arboviruses suspected, and sera generally are not sent to DHS for testing. In an attempt to stimulate detection of human SLE or WEE cases in California, communication with key hospitals and local health officials has been enhanced and specimens from suspect cases are being tested rapidly through DHS' California Encephalitis Project. Core testing for 15 agents is performed on each case and SLE, WEE, and WN are part of this core panel. In cases where patients have extensive mosquito exposure and SLE, WEE, and WN are negative, additional testing for other arboviruses is done. Over 100 cases are referred to the project each year. The rapid detection and reporting of confirmed human cases is crucial to local mosquito control agencies in planning and expediting emergency control activities to prevent additional infections.

Mosquito Control

Mosquito control in California is conducted by over 70 local agencies, including mosquito and vector control districts, environmental health departments, and county health departments. Compounds currently approved for larval and adult mosquito control in California are listed in Appendix F.

Larval Control

Control of mosquito larvae and pupae prevents mosquitoes from becoming biting female adults capable of transmitting disease, causing discomfort, and ultimately producing another generation of mosquitoes. Larval control allows for the use of target-specific agents in definable areas. For these reasons, most local mosquito control agencies target immature stages rather than the adult stage of the mosquito. Larval mosquito control has three key components: environmental management, biological control, and chemical control.

Environmental management may include water management, such as increasing the water disposal rate through evaporation, percolation, recirculation, or drainage. Controlled irrigation or the careful timing of wetland flooding for waterfowl can reduce mosquito production. Environmental management also may entail vegetation management because emergent vegetation provides food and refuge for mosquito larvae. Vegetation management strategies include the periodic removal or thinning of vegetation, restricting growth of vegetation, or controlling algal growth. Environmental management decreases habitat availability for immature mosquitoes.

Biological control entails the intentional use of natural predators, parasites, or pathogens to reduce immature mosquito numbers. Mosquitofish, *Gambusia affinis*, are the most widely used biological control agent in California. These fish are released annually in a variety of habitats, such as rice fields, small ponds, and canals.

There are several mosquito control products that are highly specific and thus have minimal impact on non-target organisms. These include microbial control agents, such as *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus*. Insect growth regulators, such as methoprene, prevent immature mosquitoes from developing into adults. Surface films are very effective against both larvae and pupae, but also may suffocate other surface breathing aquatic insects. Organophosphate pesticides are used infrequently because of their impact on nontarget organisms and the environment.

Adult Control

When larval control is not possible or has been used to the fullest extent possible, adult mosquito control may be required to suppress populations of infected mosquitoes and stem an epidemic. Adult mosquito control products may be applied either using ground-based equipment, fixed wing airplanes, or helicopters. These products include organophosphates, such as malathion and naled, and pyrethroids, such as resmethrin, sumithrin, and permethrin.

There are many factors to consider when selecting a pesticide such as (1) efficacy against the target species or life cycle stage, (2) pesticide resistance, (3) pesticide label requirements, (4) availability of pesticide and application equipment, (5) environmental conditions, (6) cost, and (7) toxicity to nontarget species, including humans.

Response Levels

The California Mosquito-borne Virus Surveillance and Response Plan is based on conditions that exist at three response levels identified as normal season, emergency planning, and epidemic. Seven risk factors that are analyzed to determine the appropriate response level include:

- Environmental conditions (snowpack, rainfall, temperature, season)
- Adult mosquito vector abundance
- Virus isolation rates from mosquitoes
- Sentinel chicken seroconversion rates
- Infection rates in wild or domestic animals
- Human cases of mosquito-borne viruses
- Proximity of detected virus activity to urban or suburban regions

Each of these factors is rated on a scale of 1 to 5, with 5 representing conditions indicative of a high risk of human infection with a mosquito-borne virus. An average rating is determined for the seven factors and is correlated with the response level as follows: normal season (1.0 to 2.5), emergency planning (2.6 to 4.0), and epidemic (4.1 to 5.0). Table 1 provides a worksheet to assist in determining the appropriate rating for each of the risk factors. The term "average" refers to averages over non-epidemic years in a specific region, such as that within the boundaries of a local mosquito and vector control district. Averages typically are determined for the preceding five-year period. The ratings given are benchmarks only, and may need to be adjusted relative to the conditions in a specific region of the state. Roles and responsibilities of key agencies involved in carrying-out the surveillance and response plan are outlined in "Key Agency Responsibilities."

Table 1. Mosquito-borne Virus Risk Assessment

Surveillance Factor	Assessment	Benchmark	Assigned
1. Environmental conditions Considers snowpack, rainfall, and ambient temperature. (Note: disease outbreaks caused by SLE can also occur during hot, dry years). In those regions where the amount of water is not environmentally dependent, agencies may substitute water management factors.	1	Snowpack, rainfall, and temperature well below average	
	2	Snowpack, rainfall, and temperature below average	
	3	Snowpack, rainfall, and temperature average	
	4	Snowpack, rainfall, and temperature above average	
	5	Snowpack, rainfall, and temperature well above average	
2. Adult mosquito vector abundance Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area.	1	Vector abundance well below average (<50%)	
	2	Vector abundance below average (50–90%)	
	3	Vector abundance average (90–150%)	
	4	Vector abundance above average (150–300%)	
	5	Vector abundance well above average (>300%)	
3. Virus isolation rate in mosquitoes Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	MIR / 1000 = 0	
	2	MIR / 1000 = 0–1.0	
	3	MIR / 1000 = 1.1–2.0	
	4	MIR / 1000 = 2.1-5.0	
	5	MIR / 1000 > 5.0	
4. Sentinel chicken seroconversion rate per 10 birds Number of chickens in a flock that develop antibodies to a particular virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration.	1	No seroconversions	
	2	One seroconversion in single flock over broad area	
	3	One seroconversion in multiple flocks in region	
	4	Two to three seroconversions per flock in multiple flocks in region	
	5	More than three seroconversions per flock in multiple flocks in region	
5. Infections in wild or domestic animals Includes only equines.	1	No equine cases over broad region	
	2	No equine cases in specific region	
	3	One equine case in broad region	
	4	One or two equine cases in specific region	
	5	More than two equine cases in specific region	
6. Human cases	1	No human cases	
	3	One human case statewide (but not within local jurisdiction or region)	
	5	One or more human cases in region	
7. Proximity to urban or suburban regions (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<u>Response Level / Average Rating:</u> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

Characterization of Conditions and Responses

Level 1: Normal Season

Risk rating: 1.0 to 2.5

CONDITIONS
• Average or below average snowpack and rainfall; average seasonal temperatures • Mosquito abundance at or below five year average (key indicator = adults of vector species) • No virus isolations from mosquitoes • No seroconversions in sentinel chickens • No equine cases • No human cases
RESPONSE
• Conduct routine public education (eliminate standing water around homes, use personal protection measures) • Conduct routine mosquito and virus surveillance activities • Conduct routine mosquito larval control • Inventory pesticides and equipment • Evaluate pesticide resistance in vector species • Ensure adequate emergency funding • Release routine press notices • Send routine notifications to physicians and veterinarians • Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training

Level 2: Emergency Planning

Risk rating: 2.6 to 4.0

CONDITIONS
• Snowpack and rainfall above average • Adult mosquito abundance greater than 5-year average (150% to 300%) • One or more virus isolations from mosquitoes (MIR / 1000 is <5) • One to three chicken seroconversions per flock of 10 birds • One or two equine cases • One human case statewide • Viral activity in small towns or suburban area • Evidence of recent infection in wild birds
RESPONSE
• Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate) • Enhance information to public health providers • Increase surveillance and control of mosquito larvae • Increase adult mosquito surveillance • Increase number of mosquito pools tested for virus • Conduct localized chemical control of adult mosquitoes • Contact commercial applicators in anticipation of large scale adulticiding • Review candidate pesticides for availability and susceptibility of vector mosquito species • Review epidemic response plan • Ensure notification of key agencies of presence of viral activity, including the local office of emergency services

Level 3: Epidemic Conditions

Risk rating: 4.1 to 5.0

CONDITIONS
• Snowpack, rainfall, and water release rates from flood control dams well above average • Adult vector population extremely high (>300%) • Virus isolates from multiple pools of mosquitoes (MIR / 1000 > 5.0) • More than three seroconversions per flock of ten birds in multiple flocks • More than two equine cases in specific region • One or more human cases in region • Virus detection in urban or suburban areas • Increased seroprevalence rates in wild bird populations or die-off of susceptible species
RESPONSE
• Conduct full scale media campaign • Alert physicians and veterinarians • Conduct active human case detection • Continue enhanced larval surveillance and control of immature mosquitoes • Broaden geographic coverage of adult mosquito surveillance • Accelerate adult mosquito control if appropriate • Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC) • Initiate mosquito surveillance and control in geographic regions without an organized vector control program • Request public health exemptions from FIFRA (40 CFR 166) and emergency tolerance exemptions (40 CFR 176) • Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer) • Determine whether declaration of a “State of Emergency” should be considered by the Governor at the request of designated county or city officials • Ensure state funds and resources are available to assist local agencies at their request • Continue mosquito education and control programs until mosquito abundance is substantially reduced and no additional human cases are detected

Key Agency Responsibilities

Local Mosquito and Vector Control Agencies

- Gather, collate, and interpret regional weather data
- Monitor abundance of immature and adult mosquitoes
- Collect and submit mosquito pools for virus isolation
- Maintain sentinel chicken flocks, obtain blood samples, and send them to laboratory
- Conduct routine control of immature mosquitoes
- Conduct control of adult mosquitoes when needed
- Educate public on mosquito avoidance
- Coordinate with local Office of Emergency Services personnel

Mosquito and Vector Control Association of California

- Coordinate purchase of sentinel chickens
- Receive, track, and disperse payment for surveillance expenses
- Coordinate surveillance and response activities among member agencies
- Maintain a standby contract with a large scale aerial pesticide applicator
- Serves as spokesperson for member agencies
- Establish liaisons with press and government officials

California Department of Health Services

- Collate adult mosquito abundance data submitted by local agencies; provide summary of data to local agencies
- Coordinate submission of specimens for virus testing
- Maintain database of all specimens tested
- Test sentinel chicken sera for viral antibodies
- Test human specimens for virus
- Distribute a weekly bulletin summarizing surveillance test results
- Send weekly surveillance results to the UC Davis interactive website
- Immediately notify local vector control agency and public health officials when evidence of viral activity is found
- Conduct epidemiological investigations of cases of equine and human disease
- Coordinate and participate in a regional emergency response in conjunction with California Office of Emergency Services
- Conduct active surveillance for human cases
- Coordinate equine and “dead bird” surveillance programs for WN and other arboviruses
- Provide oversight to local jurisdictions without defined vector-borne disease control program
- Maintain inventory of antigens and antisera to detect exotic viruses

University of California at Davis

- Conduct research on arbovirus surveillance, transmission of mosquito-borne diseases, and mosquito ecology and control
- Test mosquito pools for virus
- Provide a panel of tests for a wide range of viruses for identification of viruses from human, equine, bird, or arthropod vectors

- Maintain an interactive website for dissemination of mosquito-borne virus information and data
- Maintain inventory of antigens, antisera, and viruses to detect the introduction of exotic viruses
- Provide confirmation of tests done by local or state agencies

California Department of Food and Agriculture
- Notify veterinarians and veterinary diagnostic laboratories about WEE and testing facilities available at UCD Center for Vector-borne Disease Research
- Provide outreach to general public and livestock and poultry producers on the monitoring and reporting of equine and ratite encephalitides
- Facilitate equine and ratite sample submission from the field

California Animal Health and Food Safety Laboratory
- Screen dead birds for WNV testing
- Conduct necropsies and testing on dead crows and other birds
- Submit bird tissues to UCD and DHS for testing

Local Health Departments
- Refer human and equine specimens to DHS for further testing
- Notify local medical community, including hospitals and laboratories, if evidence of viral activity present
- Participate in emergency response
- Assist in public education

Governor's Office of Emergency Services
- Coordinate the local, regional, or statewide emergency response under epidemic conditions in conjunction with DHS via the Standardized Emergency Management System (SEMS)
- Serve as liaison with the Federal Emergency Management Agency (FEMA) in the event that a federal disaster has been declared

Centers for Disease Control and Prevention
- Provide consultation to state and local agencies in California if epidemic conditions exist
- Provide national surveillance data to state health departments

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Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of these guidelines is to standardize adult mosquito sampling and reporting procedures to provide comparable and interpretable surveillance results among collaborating mosquito control agencies in California. The four methods currently used by mosquito control agencies are: 1) New Jersey (American) light trap, 2) CDC style CO_2-baited trap, 3) gravid trap, and 4) adult resting collections. These guidelines describe: 1) a comparison of the sampling methods, 2) equipment design, 3) operation, 4) specimen processing, 5) data recording and analysis, and 6) data usage. Many mosquito traps are available commercially.

Advantages and Disadvantages of Mosquito Sampling Methods:

New Jersey Light Trap	
Pros	**Cons**
• All female metabolic states and males collected • Minimal collection effort (can be run nightly without service) • Long history of use in California	• Selective for phototaxic nocturnally active mosquitoes • Ineffective with competing light sources • Sorting time excessive because of other insects in traps • Specimens dead; useless for virus detection • Collects comparatively few specimens
CDC/EVS CO_2 Trap	
Pros	**Cons**
• Samples biting population • Collects large numbers of virus vector species • Specimens alive; suitable for virus detection • Without light, collects mostly mosquitoes thus reducing sorting time • Battery operated, portable	• Collects >50% nullipars (have never oviposited) • Must be set and picked-up daily • Dry ice cost high; availability can be a problem • Does not collect males or blooded and gravid females
Gravid Trap	
Pros	**Cons**
• Collects females that have bloodfed; may have higher infection rate • Specimens alive; suitable for virus detection • Extremely sensitive for *Cx. quinquefasciatus* in urban habitat • Bait inexpensive • Battery operated, portable	• Collects only foul-water *Culex* • Bait has objectionable odor • Must be set and picked-up daily
Resting Catches	
Pros	**Cons**
• All metabolic states collected • Minimal equipment needed • Specimens alive; suitable for virus detection • Blooded and gravid specimens can be tested to improve sensitivity of virus surveillance	• Quantification difficult due to: 1. shelter size and type 2. collector efficiency • Labor intensive

New Jersey (American) Light Trap (NJLT)

Trap specifications and components (Mulhern 1953)

1. Ten inch diameter trap tube of sufficient length to accommodate motor, fan, cone screen and killing jar. A lockable screen cage or holding strap should be added to the bottom of the trap to prevent tampering with the killing jar.
2. A 4- or 5-bladed 9.0-inch diameter fan.

3. Sealed, heavy-duty type refrigerator motor suspended by three support brackets for added stability; air discharge ≅ 450-500 cu. ft/min.
4. Hood or cone with a two- or three-point chain attachment for trap hanging.
5. One-quarter inch mesh hardware cloth over the mouth of the trap tube to preclude entry by large moths and other debris.
6. Timer or photoelectric eye to turn trap on/off. The photoelectric eye is preferred to prevent disruptions of trapping time that may occur with a timer due to power outages.
7. 25w, 110v, frosted light bulb.
8. Exterior trap color is insignificant, but underside of hood should be painted white to increase light intensity (Barr et al. 1963).
9. Killing jar with warning label containing a dichlorvos "no-pest" strip should be replaced every three months. A pint or quart jar could be used depending on the amount of insects caught.

Operation

At a minimum, trap should be located in each principal municipality of a district or have a distribution of one trap/township (36 sq. mi.) to sample the adult mosquito population within the boundaries of the district's responsibility. Correct placement of the NJLT is a critical factor in its performance as a surveillance mechanism for measuring the relative abundance of phototaxic mosquitoes. Place the traps at six-foot height. This can be done by using a metal standard, or by hanging the traps from tree limbs or roof eaves. These distances should maximize attractancy over a 360 degree radius. The trap should be placed on the leeward side of a structure or tree line to decrease the influence of wind on trap catch.

Traps should be kept away from smoke or chemical odors that may be repellent to the mosquitoes. Traps should be away from buildings in which animals are housed and not in the immediate vicinity of sentinel flocks to diminish attractancy competition. Traps should be placed away from street and security lights that may diminish attractancy of the trap bulb.

Traps should be operated from week 13 to week 43 of the calendar year for districts north of the Tehachapi Mountains and all year long for districts south of the Tehachapis. Ideally, the traps should run for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be thoroughly cleaned with a brush to remove spider webs or any other debris that may hinder airflow through the trap. A regular cleaning schedule should be maintained during the trapping season to maintain trap efficiency.

Processing

Adult mosquitoes from the NJLT collection should be sorted from the other insects in an enamel pan before being identified and counted at 10x magnification under a dissecting microscope. Counting aliquots or subsamples of all specimen samples should be discouraged because vector species may comprise only a small fraction of the total mosquito collection.

CDC style CO_2-baited trap

Trap design and components

Currently there are two types of CO_2-baited traps being used in California: CDC trap and the EVS trap (Pfuntner, 1979), which is a modification of the first. Both trap types are baited either with an insulated container holding 1-2 kg of dry ice or with a cylinder containing compressed CO_2 gas with a regulator that releases 0.5 - 1.0 liter/minute. The dry ice container or the carbon dioxide gas cylinder should be properly labeled as to their contents. Both trap styles use a screened collection bag or a modified gallon ice cream carton with tubular surgical stockinette attached to the bottom of the motor housing unit to retain the collected mosquitoes.

The CDC trap uses:

1. A 3.5" diameter plexiglass cylinder housing a 6v DC motor and a 4-blade fan.
2. Rechargeable 6v battery power source.
3. Aluminum rain shield (optional).

The EVS trap:

1. Uses a 5" diameter PVC cylinder housing a 4.5-6.0v DC motor, and a 2-blade fan.
2. Uses three 1.5v D cell batteries in series as a power source.
3. Lacks an aluminum rain shield above the trap housing.

Operation

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing. A six foot tall standard should be used to standardize trap placement; however, a convenient tree limb of sufficient height, or at times, a horizontal projection from a building or fence post will suffice. Trap location should be standardized for population and virus infection rate monitoring.

Knowledge of the host-seeking patterns of the target species is essential in determining CO_2-baited trap placement in the habitat and will enhance the catch size and therefore sampling sensitivity. *Culex tarsalis* primarily bloodfeed on birds and therefore hunt along vegetative borders and tree canopies where birds roost and nest.

Culex erythrothorax are best collected within wetland areas near dense stands of tules and cattails. In large, open breeding sources such as rice fields, CO_2-baited traps could be hung on standards on the up-wind side of the source for *Cx. tarsalis* and *Anopheles freeborni* collections. *Ochlerotatus (formerly Aedes) melanimon* and *Ochlerotatus (formerly Aedes) nigromaculis* are mammal feeders and typically hunt over open fields.

When used to supplement sentinel chickens for arbovirus surveillance, traps should be operated at different locations to enhance geographical coverage and thus surveillance sensitivity. Labor and time constraints determine the extent of sampling. When used to monitor population abundance, traps should be operated weekly or biweekly at the same fixed stations. Temperature, wind speed, wind direction, rain, etc., should be recorded, because these factors readily affect catch size. The mini-light attracts other phototaxic insects that may hinder sorting and/or damage female mosquitoes in the collection container while repelling members of the *Cx. pipiens* complex. The CO_2-baited trap should not be placed in immediate proximity to the sentinel chicken flock as it will compete with, and therefore lessen, exposure of the sentinel birds, but should be placed within 100-200m radius.

Maintenance of the traps should be performed regularly. Rechargeable 6v batteries should be charged after a night's run and rechargeable 1.5v batteries should be checked on a battery tester to determine the amount of power left to run the trap motors. Rechargeable 1.5v batteries need to be drained completely before being recharged to maintain full power capacity. Alkaline batteries need to be replaced after every use. The motors, fan blades, and interior of the trap housing should be cleaned on a regular basis.

Processing

Mosquitoes collected for arbovirus surveillance should be processed according to the procedures outlined in Appendix B. A maximum of ten pools of a species (*Cx. tarsalis*, *Ochlerotatus (formerly Aedes) melanimon*, *Ochlerotatus (formerly Aedes) dorsalis*, *Cx. pipiens, and Cx. quinquefasciatus*) should be submitted for virus testing from a given geographical location at a given time. Only live mosquitoes should be pooled for virus testing. Dead, dried specimens should be counted and discarded. Only whole specimens should be submitted; avoid including body parts (which may be from other mosquito species) or other Diptera (i.e., *Culicoides*, etc.) in the pool to prevent sample contamination. Mosquitoes collected for population monitoring are killed, identified under a dissecting microscope, and counted.

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid traps consist of a rectangular trap housing with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion mixture. The trap housing contains the motor assembly and collection chamber for gravid mosquitoes. The revised Reiter gravid trap (Reiter 1987) utilizes a 6v motor using three D cell batteries, whereas the Cummings modified gravid trap (Cummings 1992) uses a 9v motor and four D cell batteries. Both traps place the collection chamber on the inlet side of the motor so that the fan blades will not damage collected mosquitoes. The inlet height should be two inches above the surface of the hay-infusion medium to create a proper vortex.

The oviposition attractant consists of a fermented infusion made by mixing Timothy or alfalfa hay, lactalbumen, Brewer's yeast and water. The mixture should sit at room temperature for one to two days to allow fermentation and increase attractancy. New solutions should be made at least biweekly to maintain consistent attractancy.

Operation

The Reiter/Cummings gravid trap is primarily used in suburban and urban residential settings, principally for surveillance of *Culex pipiens* complex populations. The trap is placed on the ground near dense vegetation that serves as resting sites for gravid females. Specimens may be retrieved on a one to three day basis.

Processing

Culex pipiens complex females collected with the gravid trap for arbovirus surveillance should be retrieved daily and the protocol for mosquito pool submission as outlined in Appendix B should be followed. For population monitoring of the *Culex pipiens* complex, collections may be retrieved every third day. The females are killed, identified and counted before being discarded. Autogenous females may also be attracted to the gravid trap.

Adult resting collections

Trap design and operation

A flashlight and mechanical aspirator can be used to collect adult mosquitoes resting in habitats such as shady alcoves, buildings, culverts, or spaces under bridges. Highest numbers usually are collected at humid sites protected from strong air currents. Adults resting in vegetation may be collected using a mechanical sweeper such as the AFS (Arbovirus Field Station) sweeper (Meyer et al. 1983). For quantification, time spent searching is recorded and abundance expressed as number per person-hour.

Red boxes were developed to standardize collections spatially. Different researchers have used red boxes of varying dimensions. Largest catches are made in semipermanent walk-in red boxes which measure 4' x 4' x 6' (Meyer 1985). Smaller 1' x 1' x 1' foot boxes typically collect fewer specimens, but are readily portable. The entrance of the walk-in red box should be left open, draped with canvas, or closed with a plywood door. The canvas or plywood door should have a 1 or 2 ft gap at the bottom to allow entry of mosquitoes, while affording some protection from the wind and decreasing the light intensity within the box. The box entrance should not face eastward into the morning sun or into the predominant wind direction.

Processing

Mosquitoes should be anesthetized, identified under a dissecting microscope, sorted by sex and female metabolic status (i.e., empty or unfed, blood fed or gravid), and counted. Females may be counted into ten pools of approximately 50 females per site per collection date for virus monitoring (see Appendix B). Only living females should be used for arbovirus surveillance. Data on metabolic status may indicate population reproductive age as well as diapause status.

Data recording and analysis

Counts from NJLTs should be recorded on the DHS Adult Mosquito Occurrence Report Summary Form and faxed to (510) 540-3666. For comparisons of abundance over time, space, or collection methods, refer to Biddlingmeyer, 1969.

Data usage

Mosquito collections from the four sampling methods collectively can be used to:

1. Assess control efforts.
2. Compare mosquito abundance from collections with the number of service requests from the public to determine the tolerance of neighborhoods to mosquito abundance.
3. Monitor arbovirus vector abundance and minimum infection rates.
4. Determine proximity of breeding source(s) by the number of males present in collections from the NJLTs and red boxes.
5. Determine age structure of females collected by CO_2 traps and resting adult collections; such data are critical to evaluating the vector potential of the population.

References

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Loomis, E.C. and S.G. Hanks. 1959. Light trap indices of mosquito abundance: a comparison of operation for four and seven nights a week. Mosq. News 19:168-171.

Meyer, R.P., W.K. Reisen, B.R. Hill, and V.M. Martinez. 1983. The "AFS sweeper", a battery powered backpack mechanical aspirator for collecting adult mosquitoes. Mosq. News 43:346-350.

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Mulhern, T.D. 1953. Better results with mosquito light traps through standardizing mechanical performance. Mosq. News 13:130-133.

Pfuntner, A.P. 1979. A modified CO_2-baited miniature surveillance trap. Bull. Soc. Vector Ecol. 4:31-35.

Reiter, P. 1987. A revised version of the CDC gravid mosquito trap. J. Am. Mosq. Control Assoc. 3:325-327.

Appendix B. Procedures for Processing Mosquitoes for Arbovirus Detection

1. Collect mosquitoes alive and return them immediately to the laboratory. Collections should be kept humid during transport with moist toweling to prevent desiccation. Females should be offered 5-10 percent sucrose if held overnight or longer before processing.

2. Anesthetize mosquitoes by cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of SLE or WEE virus titer (Kramer et al. 1990). TEA should be used either outdoors or under a chemical hood. Collections can be knocked down outdoors using a few drops of TEA, the specimens transferred to Petri dishes, and then taken into the laboratory for processing. If refrigerated and kept humid, mosquitoes will remain alive in covered Petri dishes for one or two days without additional anesthesia.

3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that other small insects such as chironomids or *Culicoides* are not inadvertently included in the pools. Count and discard dead and dried mosquitoes. Lots of 50 females (minimum of 12 females) per pool of each vector species from each collection site are then counted. Place each mosquito pool in an individual screw-cap cryovial fitted with O-rings to prevent contact with CO_2 during transport and storage. Recommended sampling effort is ten pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from two to 20 per 1,000 females tested. Pools should be labeled sequentially starting with #1 each year after the site code. VERY IMPORTANT: POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES (USE FORM MBVS-1 TO REGISTER COLLECTION SITES - see Appendix C).

   List the site code for each pool that consists of a designated four-letter agency code followed by four digits identifying the site, i.e., KERN0001. Keep the pool numbers in sequence for the whole year regardless of the number of site codes, i.e., pool #1 may be from KERN0001, and pool #2 may be from KERN0004.

4. Freeze pools immediately at -70°C either with dry ice in an insulated container or in an ultralow temperature freezer. Pools are shipped frozen on dry ice to the UC Davis Arbovirus Research Unit for testing by an *in situ* enzyme linked immunosorbent assay (EIA). Care must be taken not to allow pools to defrost during storage or shipment, because each thaw and freeze kills approximately half the virus, and all virus will be lost if the specimens sit at room temperature.

   Davis Arbovirus Research Unit
   University of California
   Old Davis Road
   Davis CA 95616

Reference

Kramer, L.D., S.B. Presser, E.J. Houk and J.L. Hardy. 1990. Effect of the anesthetizing agent triethylamine on western equine encephalomyelitis and St. Louis encephalitis viral titers in mosquitoes (Diptera: Culicidae). J. Med. Entomol. 27:1008-1010.

MOSQUITO POOLS SUBMITTED TO UCD - 2002

AGENCY CODE: ___ ___ ___ ___ AGENCY

_____

DATE RECD. BY UCD _____

DATE TESTED _____

BULLETIN NO. _____

SITE CODE	POOL NO.	SITE NAME	DATE	TRAP TYPE	MOSQ. SPECIES	# IN POOL	WEE	SLE	CE

Form MBVS-3 (Revised 1/12/2000)

Appendix C. Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure white leghorn laying hens in March when 18 weeks of age to ensure minimal mortality during handling. Hens at this age have not yet begun to lay eggs, but should have received all their vaccinations and been dewormed. White leghorns are relatively small sized chickens, but should have well-developed combs when first bled at 22 weeks of age.
2. Ten sentinel chickens can be housed in a 3Wx6Lx3H ft coop framed with 2x2 and 2x4 inch construction lumber and screened with 1x1 inch welded wire. The site for each coop must first be registered using FORM MBVS-2 submitted to UC Davis. Coops should be at least two feet off the ground to reduce predator access, facilitate capture of the birds for bleeding, and allow the free passage of the feces through the wire floor to the ground. A single, hinged door should be placed in the middle of the coop, so that the entire coop is accessible during chicken capture. After construction, the lumber and roof should be protected with water seal. A self-filling watering device should be fitted to one end of the coop and a 25 lb. feeder suspended in the center for easy access. In exchange for the eggs, a local person (usually the home owner, farm manager, etc.) should check the birds (especially the watering device) and remove the eggs daily. If hung so the bottom is about four inches above the cage floor and adjusted properly, the feeder should only have to be refilled weekly (i.e., 100 lb. of feed per month per flock of ten birds). Therefore, if proper arrangements can be made and an empty 55 gallon drum provided to store extra feed, sentinel flocks need only be visited biweekly when blood samples are collected.
3. Band each bird in the web of the wing using metal hog ear tags and appropriate pliers. This band number, the date, and site registration number must accompany each blood sample sent to the laboratory for testing.
4. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. The bird can be immobilized by wedging the wings between the bleeder's forearm and thigh, thereby leaving the hand free to hold the head by grabbing the base of the comb with the thumb and forefinger. The comb should be "pricked" with the lancet and blood allowed to flow from the "wound" to form a drop. Collect the blood by touching the end of the pre-numbered filter paper strip (opposite from the number) to the wound. Collect several drops in this fashion to completely soak a pre-marked 3/4 inch long portion of the 3/8 inch wide filter paper strip. Place the numbered end of the strip into the slot of the holder (or "jaws" of the clothes pin) leaving the blood soaked end exposed to air dry.
5. Staple the completely dry filter paper strips through the number along the top end of a 5x8 inch card, leaving the blood soaked end free so that the laboratory staff can readily remove a standard punch sample. Write the name of the flock and the date onto the card and place it and a single flock specific data sheet into a zip lock plastic bag. It is important that blooded ends do not become dirty, wet or touch each other. VERY IMPORTANT: CHICKEN SERA MUST BE ACCOMPANIED BY SENTINEL CHICKEN BLOOD FORM (MBVS-2) OUTSIDE THE ZIP-LOCK BAG. Samples from each bleeding date then can be placed into a mailing envelope and sent to:

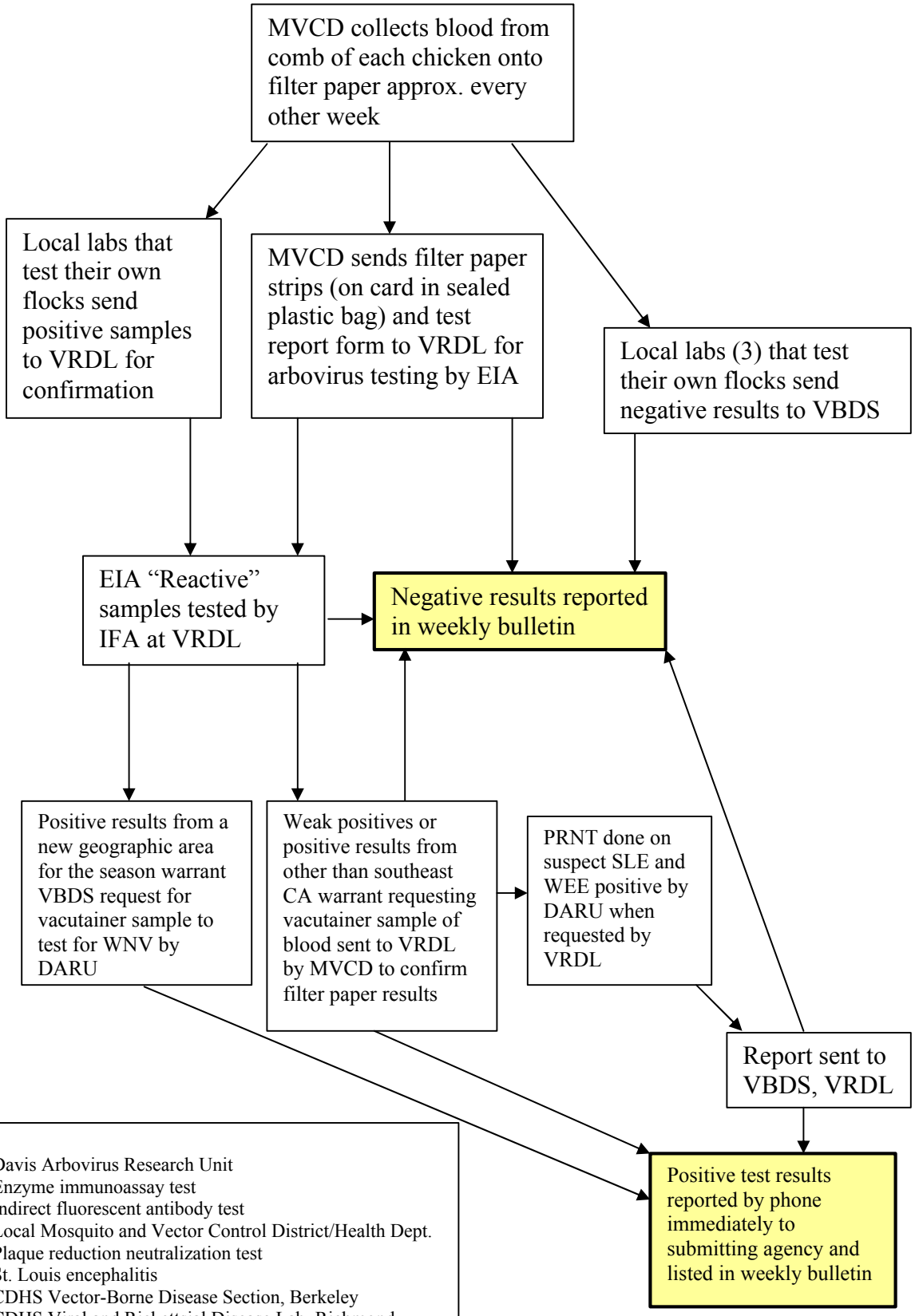
   Department of Health Services, Richmond Campus
   Specimen Receiving Unit Room B106 (ATTN: ARBO)
   850 Marina Bay Parkway
   Richmond, CA 94804

   Specimens should be mailed to arrive by Friday afternoon for testing to start the following Monday.

6. In the laboratory, a single punch is removed from the blooded end of the paper and placed into one well of a 96-well plate with 200 µl of diluent. Specimens are allowed to soak overnight and the eluate tested for WEE and SLE IgG antibody using ELISA. Positive specimens are confirmed the following day using an indirect fluorescent antibody test.

Reference: Reisen, W.K. 1995. Guidelines for Surveillance and Control of Arboviral Encephalitis in California, In: Interagency Guidelines for the Surveillance and Control of Selected Vector-borne Pathogens in California, Mosquito and Vector Control Association of California, Sacramento.

California Procedure for Testing Sentinel Chickens for the Presence of Antibodies to WEE, SLE, and WNV



Key:

DARU:	Davis Arbovirus Research Unit
EIA:	Enzyme immunoassay test
IFA:	Indirect fluorescent antibody test
MVCD:	Local Mosquito and Vector Control District/Health Dept.
PRNT:	Plaque reduction neutralization test
SLE:	St. Louis encephalitis
VBDS:	CDHS Vector-Borne Disease Section, Berkeley
VRDL:	CDHS Viral and Rickettsial Disease Lab, Richmond
WEE:	Western equine encephalitis
WNV:	West Nile virus encephalitis

2002 Surveillance for Mosquito-borne Viruses

Registration of Agencies and Sites

1. Participation of agencies

Agencies interested in participating in the statewide surveillance program for mosquito-borne viruses will place orders through MVCAC for testing of sentinel chicken blood samples and mosquito pools. MVCAC will bill the agency for the number of samples to be tested, register the agency, assign an agency code, and notify VRDL and UC of the names and codes for each registered agency.

As part of an agreement on coordination of surveillance for mosquito-borne viruses, VRDL will accept and test sentinel chicken blood samples only from those California agencies that have placed orders though MVCAC. UC Davis will accept and test mosquito pools only from those agencies that have placed orders though MVCAC.

2. Registration of sentinel flock sites and wing band numbers

Prior to submitting any sentinel chicken blood samples to VRDL, each agency must register each new flock site with UC Davis using the "SURVEILLANCE SITE REGISTRATION" form MBVS-1 (revised 1/12/2000). Blood samples sent to VRDL must be accompanied by the form "SENTINEL CHICKEN BLOOD – 2002" (MBVS-2, revised 1/12/2000) for each flock site.

Fill out a MBVS-2 form for each site and include a four digit numeric code for the site along with the wing band numbers of chickens placed at that site. Also include the date the chickens were bled. VRDL will cross check the agency and site code numbers before testing the samples.

VRDL will test samples only if they are accompanied by the appropriate 2002 form which includes the registered agency code (assigned by MVCAC), the registered site code (assigned by you), and, for blood samples, the wing band numbers assigned to that site.

3. Registration of mosquito sampling sites

Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by faxing a copy of the "SITE SURVEILLANCE REGISTRATION 2001" form to (530) 752-1537 (UC Davis) or e-mailing it to klorenzen@ucdavis.edu at the same time the pools are shipped to UC Davis. UC will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form (MBVS-3, revised 12/11/01), including your agency code, your site code for the site and geographic coordinates. If you are unable to determine the geographic coordinates, please provide a map to UC Davis showing the location of each site and its site code.

The geographic coordinates will be used to generate computer maps that will show all registered sites and test results for each site each week. Also, as part of a collaborative effort, UC Davis will be generating up-to-date maps from the weekly results for inclusion on Vector Web site: http://vector.ucdavis.edu/.

4. If there are any questions, please contact Marty Castro, Vector-Borne Disease Section at (707) 576-2733 or arbovirus@dhs.ca.gov.

SURVEILLANCE SITE REGISTRATION 2002

AGENCY CODE: **SITE CODE**:____ ____ ____ ____
(Must be numeric, no letters)

THIS FORM IS FOR REGISTRATION OF SITES USED FOR SENTINEL CHICKENS, COLLECTION OF MOSQUITOES FOR VIRUS TESTING, OR ABUNDANCE REPORTS*

_____ _____ _____
Submitting agency **County in which site is located** **Elevation in feet**

Latitude _____°_____' _____ "North (Degrees, minutes, seconds)
Longitude _____° _____' _____"West (Degrees, minutes, seconds)

Site is located _____ miles in a _____ direction from _____
(Example = NW) (City or place shown on common maps)

BRIEF DESCRIPTION OR NAME OF THE SITE USED BY SUBMITTING AGENCY:

THINGS OR CONDITIONS WITHIN 100 FEET OF THE SITE (CIRCLE THOSE THAT APPLY)

HARBORAGE	MOISTURE	HUMAN PRESENCE	ANIMALS/PETS
Buildings	Lawn or garden irrigation	Occupied dwelling	Large livestock
Trees	Agricultural irrigation	Mostly daytime presence	Rabbits
Shrubs/brush	Stream, canal, ditch	Mostly night time presence	Dogs/cats
Tall grass/weeds	Lake, pond or marsh	Usually unoccupied	Rodents
Rodent burrows	Dairy drain		Wild bird nests/roosts
Culvert or bridge	Septic tank overflow	LIGHT SOURCES	Sparrows/finches
Lumber or junk	Water trough	Dwelling or other building	Poultry
Tire pile	Tree holes	Street or yard lighting	Emus or ostriches
Other	Other	None	Other animals

LANDSCAPE OR CONDITIONS WITHIN ONE MILE OF THE SITE (CIRCLE THOSE THAT APPLY)

LANDSCAPE	AGRICULTURE	OTHER USES	TOPOGRAPHY
Forest	Orchards	Houses	Flatlands
Savannah	Vineyards	Apartments	Hill (<2,500')
Meadow	Row crops	Commercial	Mountains (>2,500')
Riverine	Rice	Park, golf course, cemetery	
Wetlands, fresh water	Irrigated pasture	Airport or industrial	URBANIZATION
Wetlands, saline	Rangeland	Right-of-way	None-rural
Desert	Poultry ranch	Other	Suburban
Other	Dairy drain, oxidation pond		Urban
	Feed lot		

*TO REGISTER A SITE, COMPLETE AND FAX TO: UC DAVIS AT (530) 752-1537.

Once registered, the same number should be used for a site year after year. If a site is relocated, the old number should be retired and a new one assigned and faxed to (530) 752-1537or e-mailed to klorenzen@ucdavis.edu.

Form MBVS-1 (Revised 1/12/2000)

SENTINEL CHICKEN BLOOD - 2002

PLEASE DO NOT PLACE THIS SHEET INSIDE THE ZIPLOCK BAG

VRDL PAGE NUMBER

REGISTERED AGENCY CODE: ___ ___ ___ ___ *SITE CODE ___ ___ ___ ___

Name of Agency:_____

Name of Site:_____Nearest City or Place:_____

DATE BLED:___/___/___ BLED BY:_____

CONTACT NAME:_____ Telephone (___) ____-_____

NAME OF ALTERNATE:_____ Telephone (___) ____-_____

WING BAND NUMBER IN SEQUENCE	REMARKS OR STATUS ("New" dead, missing, etc.) For new birds to flock, list the number and state "new bird"	WEE	SLE

Remarks:(After bird has been reported dead, put band number and list as "old dead" or "old missing" in this space)

Date received by VRDL: ____/____/____. Tested: ____/____/____. Reported to agency contact:____/____/____
List all birds that have been in the flock.

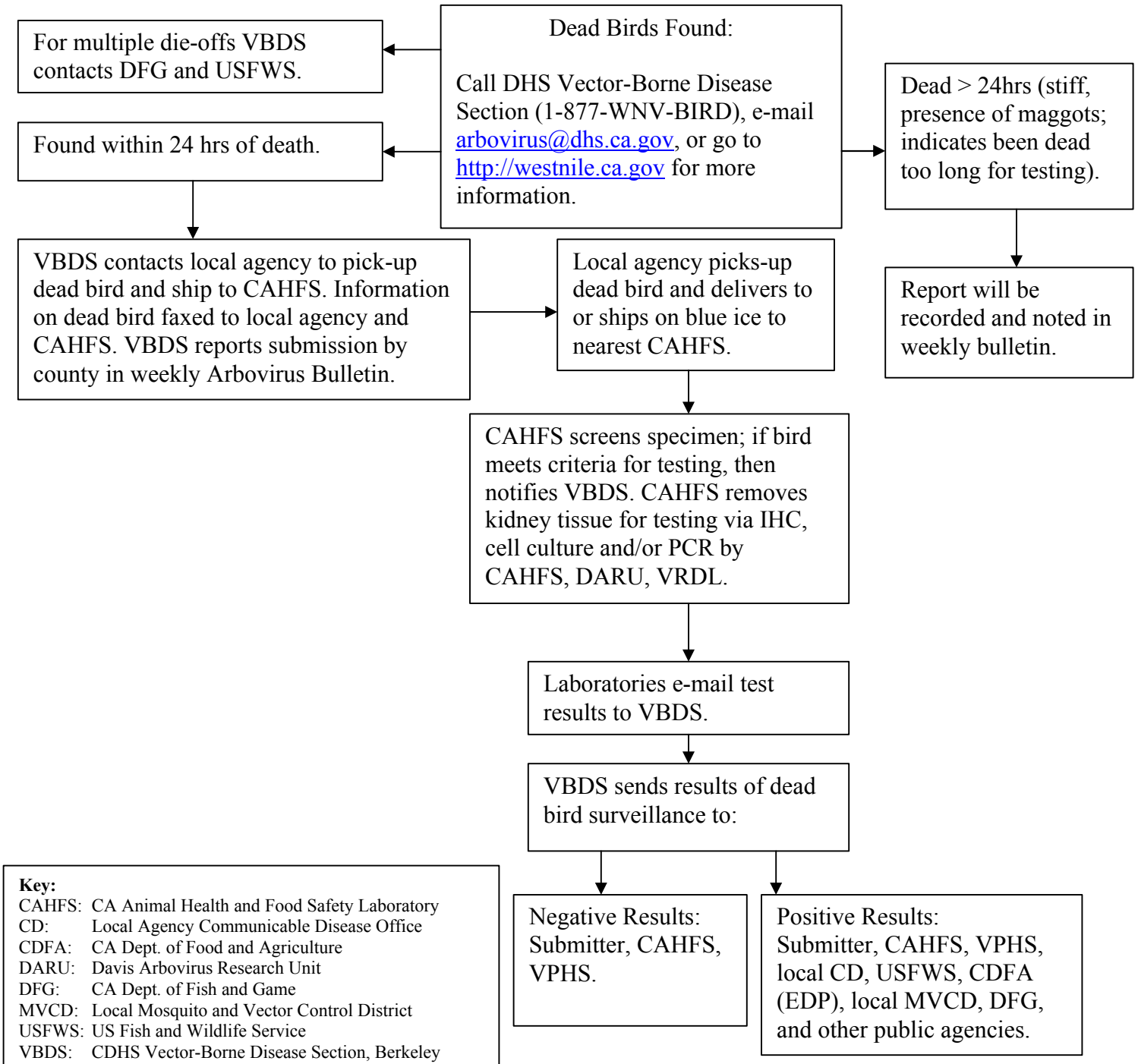
A COPY OF THIS FORM MUST ACCOMPANY ALL SHIPMENTS OF CHICKEN BLOOD TO VRDL. ANY FUTURE SHIPMENTS FROM THIS SITE MUST USE THE SAME SITE CODE. TO REGISTER A SITE, FAX A COPY OF THE SITE REGISTRATION FORM (MBVS-1) TO (530) 752-1537, UC DAVIS.

Form MBVS-2

Appendix D: Procedures for Testing Dead Birds

In mid-2000, DHS initiated a dead bird surveillance program in collaboration with other public agencies. DHS annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. Dead birds are reported to DHS, shipped to a California Animal Health & Food Safety Laboratory for screening and removal of kidney tissue, which is then sent to the UC Davis Arbovirus Research Unit for WNV viral isolation.

California Department of Health Services (DHS)
West Nile Surveillance Program
For Testing Dead Birds: 2002



Key:

CAHFS:	CA Animal Health and Food Safety Laboratory
CD:	Local Agency Communicable Disease Office
CDFA:	CA Dept. of Food and Agriculture
DARU:	Davis Arbovirus Research Unit
DFG:	CA Dept. of Fish and Game
MVCD:	Local Mosquito and Vector Control District
USFWS:	US Fish and Wildlife Service
VBDS:	CDHS Vector-Borne Disease Section, Berkeley
VPHS:	CDHS Veterinary Public Health Section, Sacramento (916) 327-0332
IHC:	Immunohistochemistry

Revised 7/10/02

State of California—Health and Human Services Agency

Department of Health Services

DIANA M. BONTÁ, R.N., Dr. P.H.
Director

GRAY DAVIS
Governor

Date: June 4, 2002

To: Colleagues and public or private agencies involved with West Nile virus surveillance

From: Vector-Borne Disease Section, Veterinary Public Health Section, and
Viral and Rickettsial Disease Laboratory Branch

Subject: 2002 West Nile Virus Surveillance Program: Dead Birds

Report crows, ravens, magpies, jays and hawks that have been dead for less than 24 hours in your area by calling the California Department of Health Services (DHS) West Nile Virus Hotline at 1-877-WNV-BIRD, which is staffed from 8am-4pm weekdays. On a weekly basis, DHS will summarize the California and U.S. West Nile surveillance programs at http://westnile.ca.gov. Although DHS will accept reports of deaths among other bird species, all concerns about wildlife health should always be reported to the Department of Fish and Game.

Background. West Nile (WNV) virus was first identified in the United States in the summer of 1999 following an outbreak of encephalitis in New York resulting in 62 human cases. In 2000, there were 21 human cases and 4,139 WNV infected birds reported. In 2001, there were 66 human cases and 7,114 infected birds reported. WNV virus activity has been documented in 27 states, the District of Columbia and the Cayman Islands. Although WNV has not been detected in California, the virus can potentially be introduced into our state through interstate or international movement or transport of infected birds, mosquitoes, mammals, or by an infected traveler. Information from the eastern half of the United States indicates that dead bird reporting is an important component of an early warning system to detect WNV activity in a new geographic area. Significant mortality among crows in most states preceded the outbreak of WNV encephalitis among humans and equines in both 1999 and 2000.

California Program. This is the third year of the DHS collaborative dead bird surveillance program for WNV. Cooperating agencies include: Centers for Disease Control and Prevention (CDC), Davis Arbovirus Research Unit (DARU), California Animal Health and Food Safety Laboratory System (CAHFS), Department of Fish and Game (DFG), and the U.S. Fish and Wildlife Services (USFWS). Enclosure (1) is the revised algorithm of the dead bird surveillance program and the roles of the above agencies.

DHS will screen and approve dead birds for WNV and other mosquito-borne infections on a case-by-case basis. Submitters must consult with DHS prior to collecting or shipping birds for testing. Collecting or transporting wildlife including dead birds requires permits from DFG and USFWS; these permits have been acquired by DHS. Copies were sent to those agencies which will pick-up dead birds and ship to CAHFS. Enclosure (2) is the Dead Bird Submission Instructions for local agencies listed on the permits. Since wild birds may carry diseases that are infectious to humans, only trained persons should handle dead birds using appropriate precautions. DHS will coordinate the shipment pick-up and cover the cost for shipping and laboratory testing.

Approved bird carcasses will be sent to the nearest CAHFS laboratory for screening and removal of kidney tissue. The excised kidney tissue will be forwarded to DARU for viral assays. DHS will report the first results back to submitters and participating agencies within 2-3 weeks. An e-mail weekly summary report of WNV and other

mosquito-borne disease testing in California is available at http://westnile.ca.gov under "News". The California WNV brochure is also available on the website.

Your participation in this collaborative surveillance effort will ensure that we are collectively responding to the threat of WNV, and ultimately reducing the risk of human infection in California. If you should require additional information or have any questions, please contact the DHS Vector-Borne Disease Section at (877) WNV-BIRD, the Veterinary Public Health Section at (916) 327-0332 or e-mail us at arbovirus@dhs.ca.gov.

Enclosures

Dead Bird Submission Instructions for Local Agencies
California West Nile Virus (WNV) Dead Bird Surveillance Program
California Department of Health Services (DHS)
Division of Communicable Disease Control

Dead Bird Reporting and Submission Instructions for Local Agencies

When your agency receives a call from the public about a dead crow, raven, magpie, jay, or hawk, or one of your staff finds a dead bird, please follow one of these choices of action:

Immediately refer them to the DHS Hotline at 1-877-WNV-BIRD (877-968-2473). **DHS will assess the suitability of the dead bird for testing and contact your agency only if the bird is approved for pick-up. The WNV Hotline is monitored 8am-4pm Monday through Friday. Any dead birds sent without prior notification will not be tested.**

-OR-

Refer the caller to the person(s) in your agency who has been trained in the WNV Hotline protocol. If your agency would like to train employees in procedures related to screening birds for testing please call Stan Husted at 1-510-540-2712. He will discuss with you our protocol on responding to calls and maintaining records.

Once the dead bird submission is approved, DHS will arrange for the pick-up of the bird to be shipped from your agency to the nearest California Animal Health and Food Safety Laboratory (CAHFS). Shipping and testing expenses will be paid by DHS. The lab will do screening tests, remove kidney tissue, and forward the tissue to the Davis Arbovirus Research Unit (DARU) for viral assay.

To ensure the proper condition of specimens for testing and to comply with regulations for shipping diagnostic specimens, please follow these instructions.

Bird Carcasses

- Only dead birds can be picked-up according to our permit.
- Do not touch the carcass with bare hands: wear rubber or latex gloves when picking up and handling it. If gloves are not available use a plastic bag turned inside out to pick-up the bird.
- Only agencies listed under the permit issued to DHS from the California Department of Fish and Game and U.S. Fish and Wildlife Service are authorized to pick-up dead birds. The agencies covered include local mosquito abatement districts, some animal control departments, and other designated agencies.
- Collect freshly dead birds (dead < 24 hours). If maggots are present or the body is stiff, the carcass is unacceptable. Viruses usually die 48 hours after the bird dies. Decomposed or scavenged carcasses cannot be tested.
- **If upon pick-up the carcass is found to be unacceptable (wrong species or decomposed), please collect the bird and dispose of it by placing it inside a double bag (tie or zip lock) and place it in a secure garbage can or dumpster.** California Department of Fish and Game and the U.S. Fish and Wildlife Service prefer that your burn or bury the carcass, but disposing of it in a dumpster is acceptable. **Immediately call DHS and notify them that the bird will no longer be tested so that we can remove the bird from the "tested" category.**
- Place each bird carcass into a plastic bag and secure it inside a second plastic bag, then zip lock it shut. **Double bagging prevents cross contamination and leakage. There should always be two bags separating the bird from documents/ labels that accompany it during shipping.**
- **Pack the bird carcass with blue ice packs (do not use dry ice unless specifically requested by DHS).**
- **Enclose the shipping document into a SEPARATE ZIP-LOCK BAG. Information includes a return-address label, so your box can be returned, and a copy of the DHS form (with the dead bird number) that was faxed**

to you by DHS after you reported the bird. CAHFS prefers you put this separate zip-lock bag inside the outer bag containing the dead bird.

- Ship bird carcass in a hard-sided plastic cooler or a styrofoam cooler placed in a cardboard box. Unprotected styrofoam containers may break into pieces during shipment. **Notify DHS to arrange for a carrier pick-up to ship Monday through Thursday. This guarantees arrival at CAHFS before the weekend.** If the carcass is fresh and needs to be shipped on Friday, call 1-877-WNV-BIRD to make special arrangements or to obtain instruction on storage until shipment on Monday.

- Label the outside of the package with the words **Diagnostic Specimens ATTN: WNV** above the designated CAHFS address.

Dead Bird Shipping List

In preparation for the arrival of West Nile Virus, please verify that your district has the following items:

Self-Adhesive Dry Ice Shipping labels (In the unlikely event you will need to ship on dry ice, some labels have been enclosed, but you can use them for your mosquito pools).

- CAHFS Addresses (see below)
- Bubble wrap or crumpled newspapers
- Rubber or Latex Gloves
- Dead Bird Shipping Boxes
  - inner zip-lock bag
  - outer zip-lock bag
  - Inner Styrofoam box
  - Outer Cardboard box
- Brown Shipping Paper
- DHS Phone Number: 877-WNV-BIRD (manned 8am-4pm weekdays).

CAHFS Central Laboratory (530) 752-9709
ATTN: WNV
Dr. Leslie Woods
University of California, Davis
West Health Science Drive
Davis, CA 95616

CAHFS San Bernardino (909) 383-4287
ATTN: WNV
Dr. Deryck Read
105 West Central Avenue
San Bernardino, CA 92412

CAHFS Fresno (559) 498-7740
ATTN: WNV
Dr. Richard Chin
2789 South Orange Avenue
Fresno, CA 93725

CAHFS Turlock (209) 634-5837
ATTN: WNV
Dr. Bruce Charlton
P.O. Box 1522
Fulkerth & Soderquist Road
Turlock, CA 95381

Appendix E: Procedures for Testing Equines

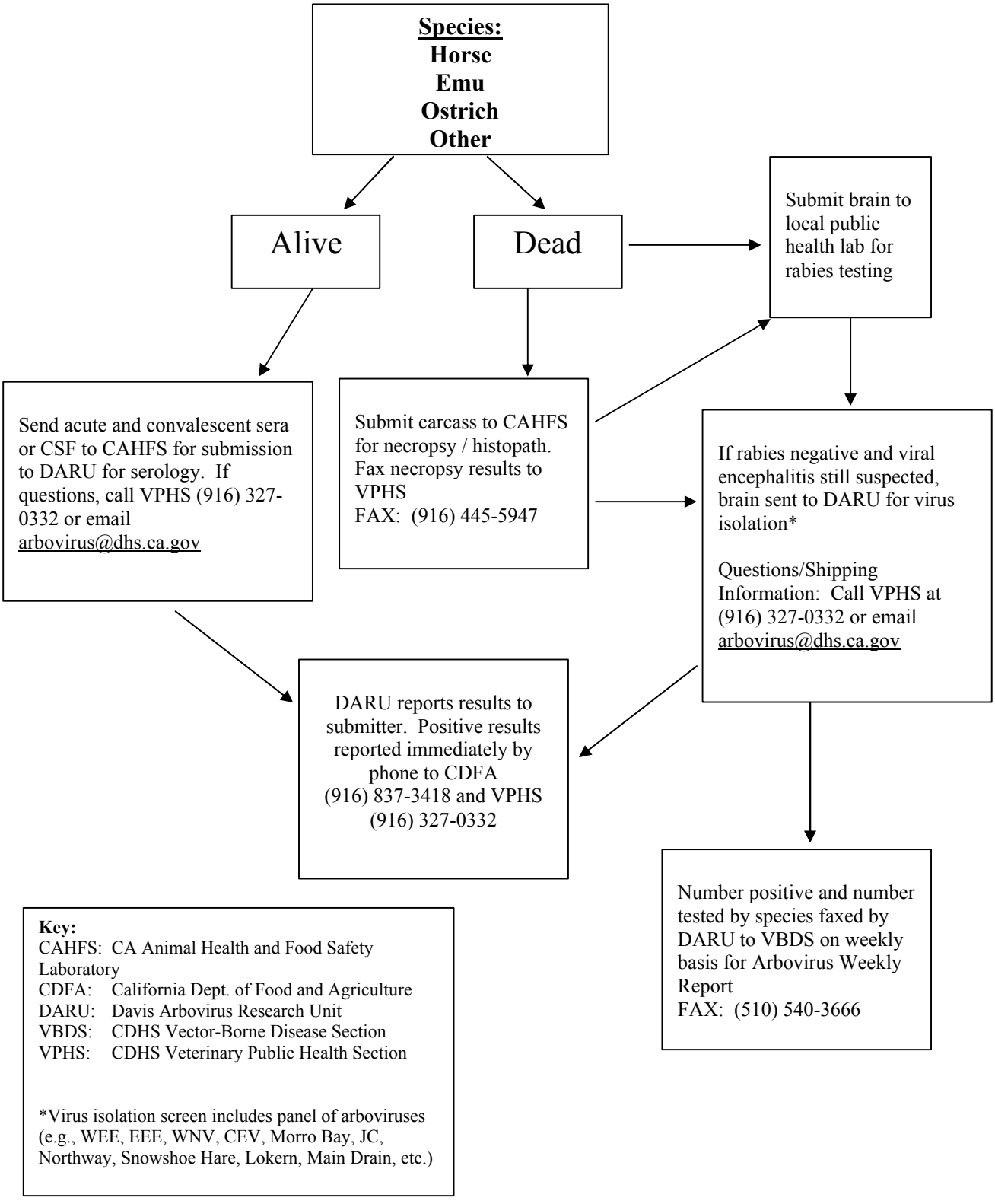
The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) have a well-established passive surveillance program for equine encephalomyelitis. Equine encephalomyelitides are legally reportable to CDFA by veterinarians and diagnostic laboratories pursuant to Section 9101 of the Food and Agricultural Code. Venezuelan equine encephalitis is an emergency animal disease that must be reported to CDFA by telephone within 24 hours.

This appendix contains a copy of the mailing sent to veterinarians every spring to inform them about the California Equine Arbovirus Surveillance Program. The mailing includes a case definition for equine encephalomyelitides and instructions for specimen collection and submission. The mailing is distributed to approximately 1,200 practitioners, equine organizations, and other interested parties. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System's (CAHFS) five regional branches, the University of California at Davis (UCD) School of Veterinary Medicine's Veterinary Medical Teaching Hospital, and other laboratories or individual veterinarians. Equine testing is done by the UCD Center for Vector-Borne Disease Research, Arbovirus Research Unit (DARU). Tests include virus isolation and identification of a large panel of arboviruses (WEE, EEE, WN, California encephalitis, Morro Bay, Jamestown Canyon, Northway, Snowshoe Hare, Lokern, Main Drain) and serology using the plaque reduction neutralization test (WEE, EEE, WN, SLE). All fatal cases of equine encephalitis are first tested for rabies at the local public health laboratory. An algorithm outlining the protocol for specimen submission and reporting is available for participants in the program.

Outreach is an important component of the program. DHS and CDFA have developed and distributed educational materials concerning the diagnosis and reporting of arboviruses in equines. DHS and CDFA work closely with equine veterinary referral centers, the California Horse Racing Board, and other interested parties to improve surveillance and reporting of suspect cases of equine encephalomyelitides.

Additional information on WN virus for veterinarians and horses owners, including a fact sheet on the equine West Nile virus vaccine, is available at CDFA's website: http://www.cdfa.ca.gov/ahfss/ah/wnv_info.htm.

Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



STATE OF CALIFORNIA

DEPARTMENT OF FOOD AND AGRICULTURE
ANIMAL HEALTH AND FOOD SAFETY SERVICES
ANIMAL HEALTH BRANCH
1220 N STREET, ROOM A-107
SACRAMENTO, CA 95814
(916) 654-1447

DEPARTMENT OF HEALTH SERVICES
DIVISION OF COMMUNICABLE DISEASE
VETERINARY PUBLIC HEALTH SECTION
601 North 7th Street, M/S 486
P. O. BOX 942732
SACRAMENTO, CA 94234-7320
(916) 327-0332

April 2002

TO: California Veterinarians in Large Animal Practice
Public Health Laboratory Directors
Local Health Officers
Public Health Veterinarians
Animal Health Branch Personnel
Interested Parties

SUBJECT: **SURVEILLANCE AND REPORTING OF ARBOVIRAL ENCEPHALITIS VIRUSES IN HORSES AND RATITES**

The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) provide **free diagnostic testing for arboviral encephalitis viruses**. These include western equine encephalitis (WEE), eastern equine encephalitis (EEE), and West Nile (WN) viruses. These diseases may affect horses, ratites (ostriches, emus, rheas, etc.), humans, and other birds and mammals. Therefore, your continued support of the surveillance program in California is important to both human and animal health. **Equine specimen submission instructions are provided in Attachment A**. Ratite case submissions should be coordinated through a California Animal Health and Food Safety (CAHFS)* Laboratory in your area (see attachment).

Veterinarians are often the first to detect the emergence of zoonotic diseases such as WN virus. There were 738 cases of clinical cases of WN virus reported in 20 states in 2001. Of 470 horses for which an outcome has been reported, 156 (33.2%) died or were euthanized. To date, this disease has not been found in California. However, the potential exists for its introduction this year via infected mosquitoes, birds, or mammals. Therefore, continued vigilance on the part of veterinarians, public health officials, and animal keepers is critical. The decision to vaccinate for WN virus should be based on a risk assessment analysis between the horse owner and their veterinary practitioner. Although challenge studies have not been done on this new vaccine, it reportedly takes about 6 weeks from the time of the first of two required shots for the production of measurable antibodies.

There have been high levels of WEE virus activity in sentinel chickens and mosquitoes in recent years. Therefore, clients should be encouraged to vaccinate their horses for WEE virus before the encephalitis season begins in late May or June. It is important to emphasize that once a horse has developed encephalitis, vaccination provides no benefit.

Furthermore, due to the possibility that vaccination will interfere with diagnostic tests, combination equine encephalitis vaccines should not be used in suspect cases.

Your participation in this important public health program is greatly appreciated. For more information on WN virus and other equine encephalitis viruses, please visit our website at http://westnile.ca.gov. If you require additional information, please contact your District Office of the CDFA, Animal Health Branch (see attachment) or the Veterinary Public Health Section of DHS at (916) 327-0332.

Importantly, we are creating a WN e-mail distribution list. If you wish your hospital/clinic's name to be included, please forward the e-mail address with subject line "WN" to: Lpritchard@cdfa.ca.gov

Kenneth L. Thomazin, D.V.M
Chief
Animal Health Branch

Michele Jay, D.V.M., M.P.V.M.
Acting Chief
Veterinary Public Health Section

Attachments

cc: Alex Ardans, D.V.M., M.S., Director
California Animal Health and Food Safety Laboratory System

Tom Scott, Ph.D., Director
Davis Arbovirus Research Unit
Center for Vector-Borne Disease Research
University of California at Davis

* See CAHFS attachment for locations and addresses

SURVEILLANCE CASE DEFINITION FOR CONFIRMED WEST NILE VIRUS INFECTION IN EQUINES

NOTE: A HORSE WITH SIGNS OF ENCEPHALITIS MAY HAVE RABIES – TAKE PROPER PRECAUTIONS

Confirmed Case:

A horse with compatible clinical signs including ataxia (stumbling, staggering, wobbly gait, or incoordination) or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, blindness, lip droop/paralysis, teeth grinding, acute death.

Plus one or more of the following:

- Isolation of West Nile (WN) virus from tissues[1]
- An associated 4-fold or greater change in plaque-reduction neutralization test (PRNT) antibody titer to WN virus in appropriately timed[2], paired sera
- Detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or cerebrospinal fluid (CSF) and an elevated titer (1:10 or greater to WN virus antibody by PRNT in serum;
- Detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or CSF and a positive polymerase chain reaction (PCR)[3] for WN virus genomic sequences in tissues[1]
- Detection of both IgM antibody to WN virus by IgM-capture ELISA in serum or CSF and a positive immunohistochemistry (IHC) for WN virus antigen in tissue;
- Positive IHC for WN virus antigen in tissue and a positive PCR[3] for WN virus genomic sequences in tissues.

Probable Case[4]:

Compatible clinical signs plus one of the following:

- Detection of IgM antibody to WN virus by IgM-capture ELISA in serum or CSF, but no elevated titer (negative at 1:10) to WN virus antibody by PRNT in serum
- No positive PCR[3] for WN virus genomic sequences tissues, and no positive IHC for WN virus antigen in tissue;
- Positive PCR[3] for WN virus genomic sequences in tissues;
- Positive IHC for WN virus antigen in tissue.

Suspect Case[4]:

Compatible clinical signs

[1] Preferred diagnostic tissues from equine are brain or spinal cord; although tissues may include blood or CSF, the only known reports of WN virus isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.
[2] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.
[3] For horses it is recommended that rt-nested polymerase chain reaction assay be used to maximize sensitivity of the test (Emerg Infect Dis. 2001 Jul-Aug; 7(4):739-41)
[4] An equine case classified as a suspect or probable case should, if possible, undergo further diagnostic testing to confirm or rule out WN virus as the cause of the clinical illness

Assumptions on which case definition is based:

- Antibody in serum may be due to vaccination or a natural exposure; additional testing must be done to confirm WN virus infection in a vaccinated horse.
- IgM-capture ELISA testing may be slightly non-specific; cross-reactions to closely related flaviviruses (e.g., SLE virus) may occur.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WN virus or a closely related flavivirus has occurred, very likely within the last three months.
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WN virus; it is possible that clinical signs may be present in an equine before a serum PRNT is positive.
- Neutralizing antibody detected in serum by PRNT indicates past exposure to WN virus; equines exposed to WN virus prior to 2002 may test positive for neutralizing antibody by PRNT.

PLEASE CONTACT CDFA OR DHS TO DISCUSS WEST NILE VIRUS TEST RESULTS FROM PRIVATE DIAGNOSTIC LABORATORIES

Attachment A: Protocol for Submission of Laboratory Specimens for Equine Neurological Disease Diagnosis and Surveillance April 2002

1. Specimen collection and submission:

A. Blood

- Acute sample (5-10 ml) / no later than 7 days after onset
- Convalescent sample (5-10 ml) / 14-21 days after onset

Red top tubes of whole blood or serum (no preservatives or anticoagulants) should be submitted at ambient temperature to the California Animal Health and Food Safety (CAHFS) Laboratory* in your area. Do not freeze whole blood.

B. Brain

- Submission of the intact head is preferable because: 1) brain is better preserved (anatomically and virus titer) when left in the skull during transport, 2) specimens will be ruined if removal is not done correctly, and 3) brain removal in field conditions may increase the risk of exposure to rabies.
- **The intact head should be chilled immediately after removal. Submit it to a CAHFS Laboratory* in your area as quickly as possible.** Prepare a leak proof insulated transporting container with "cold packs" to keep the specimen at 4^{o} C while in transit. *When it is impossible for the CAHFS Laboratory to receive the chilled intact head within 48 hours, the submission protocol should be coordinated with the lab.*
- Specimens will then be forwarded by CAHFS to 1) a Public Health Laboratory to confirm or rule out rabies, and 2) The California Vector Borne Disease Laboratory (CVBRD) for arboviral testing.

C. Other specimens for differential neurological diagnoses

- Protocol for submission of these specimens may be coordinated through CAHFS*.

2. Submission forms: Complete and include the transmittal forms supplied by the CAHFS. See attached sample or download the form from their website: http://shpinx.ucdavis.edu The submittal form for each specimen should be placed in a leak proof plastic bag and attached to the corresponding container.

3. Shipment: Check with the CAHFS Laboratory in your area for assistance with shipping regulations governing the transportation of infectious materials.

* See CAHFS attachment for locations and addresses

Appendix F. Compounds Approved for Mosquito Control in California

Label rates and usage vary from year to year and geographically; consult your County Agricultural Commissioner and the Department of Fish and Game before application. Examples of products containing specific active ingredients are provided below, but this is not an inclusive list nor constitutes product endorsement. For more information on pesticides and mosquito control, please refer to the Environmental Protection Agency (EPA) Web site:

http://www.epa.gov/pesticides/factsheets/skeeters.htm

Larvicides:

1. *Bacillus thuringiensis israelensis* (BTI: e.g. Vectobac, Teknar)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not persist well in the water column.
2. *Bacillus sphaericus* (e.g. Vectolex)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not work well on all species. May persist and have residual activity in some sites.
3. IGRs (Insect Growth Regulators)
   a. Methoprene (e.g. Altosid)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Works best on older instars. Some populations of mosquitoes may show some resistance.
   b. Diflurobenzamide (e.g. Dimilin)
   Use: Impounded tailwater, sewage effluent, urban drains and catch basins.
   Limitations: Cannot be applied to wetlands, crops, or near estuaries.
4. Larviciding oils (e.g. Golden Bear 1111, BVA Chrysalin)
   Use: Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   Limitations: Consult California Department of Fish and Game for local restrictions.
5. Monomolecular Films (e.g. Agnique MMF)
   Use: Most standing water including certain crops.
   Limitations: Does not work well in areas with unidirectional winds in excess of ten mph.

Adulticides:

1. Organophosphate compounds
   Note: Many *Cx. tarsalis* populations in the Central Valley are resistant to label OP application rates.
   a. Malathion (e.g. Fyfanon)
   Use: May be applied by air or ground equipment over urban areas, some crops including rice, wetlands.
   Limitations: Paint damage to cars; toxic to fish, wildlife and bees; crop residue limitations restrict application before harvest.

b. Naled (e.g. Dibrom, Trumpet EC)
   Use: Air or ground application on fodder crops, swamps, floodwater, residential areas.
   Limitations: Same as malathion.

2. Pyrethrins (natural pyrethrin products: e.g. Pyrenone Mosquito Spray, Pyrocide)
   Use: Wetlands, floodwater, residential areas, some crops.
   Limitations: Do not apply to drinking water, milking areas; may be toxic to bees, fish, and some wildlife. Some formulations with synergists have greater limitations.

3. Pyrethroids (synthetic pyrethrin products containing resmethrin or permethrin: e.g. Scourge)
   Use: All non-crop areas including wetlands and floodwater.
   Limitations: May be toxic to bees, fish, and some wildlife; avoid treating food crops, drinking water or milk production.